elusive, in part, due to the often small size and transient nature of functional membrane heterogeneities in cellular membranes. Recently, we introduced a powerful model membrane platform that allows the thorough analysis of membrane protein sequestering and oligomerization in well-defined heterogeneous lipid environments in the absence of artificial crosslinking agents using single molecule-sensitive confocal fluorescence intensity analysis paired with a photon counting histogram (PCH) method (1). By comparing the sequestration behavior of integrins ($\alpha v\beta 3$ and $\alpha 5\beta 1$) and urokinase receptors in such a model membrane platform, here we provide insight into the potentially distinct mechanisms of protein sequestration of transmembrane and GPI-anchored proteins in biological membranes. Specifically, the distinct protein sequestration behavior of both types of membrane proteins is illustrated in terms of bilayer asymmetry, ligand binding, and bilayer cholesterol content.

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2536-Pos Board B228

Monolayer Spontaneous Curvature of Raft-Forming Membrane Lipids

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We have derived the monolayer spontaneous curvatures J_0 for a series of phosphatidylcholines, phosphatidylethanolamines, sphingomyelin, and cholesterol as a function of temperature. For this purpose lipids were transferred into inverted hexagonal phases using dipalmitoyl phosphatidylethanolamine according to established protocols (see, e.g. [1-3]) and investigated by small angle x-ray scattering. The neutral plane for each lipid, which defines J_0, was determined from the corresponding electron density maps. Spontaneous curvatures of phosphatidylethanolamines and cholesterol were found to be at least a factor of two more negative than those of phosphatidylcholines, whose J_0 is closer to zero. Interestingly, a significant positive $J_0 = +0.1\ \mathrm{nm}^{-1}$ was retrieved for dipalmitoylphosphatidylcholine at 25°C. Spontaneous curvatures in general were found to become more negative with increasing temperature with thermal expansion coefficients that ranged between $-1\cdot10^{-3}$ to $-3\cdot10^{-3}\ (^{\circ}\mathrm{C\,nm})^{-1}$ for most investigated lipids. The data allowed us to estimate the monolayer spontaneous curvatures of ternary lipid mixtures showing liquid ordered / liquid disordered phase coexistence. We report selected spontaneous curvature phase diagrams and discuss effects on protein insertion and line tension.

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2537-Pos Board B229

Hybrid and Nonhybrid Lipids Exert Common Effects on Membrane Raft Size and Morphology

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Cell membranes perform multiple functions that may be facilitated by the lateral organization of lipids and proteins into nanoscale compartments, termed membrane rafts. Understanding the origins of raft domains and the physicochemical mechanisms that control their finite small size is important for elucidating their functional significance and manipulating their properties. However, it has proven difficult to characterize rafts in cells due to the chemical complexity of biological membranes, and various models have been advanced based on theory and experiments of representative model membranes. In one popular model, a special role in the domains existence and properties has been postulated for chain-asymmetric or hybrid lipids having a saturated sn-1 chain and an unsaturated sn-2 chain. It was proposed that these lipids align in a preferred orientation at the boundary of ordered and disordered phases, lowering the interfacial energy and thus reducing domain size. Such a unique "line-active" role for hybrid lipids is an appealing explanation for nanoscopic rafts, as animal cell membranes contain few symmetric low-melting lipids but an abundance of hybrid lipids. We present data from small-angle neutron scattering and fluorescence techniques demonstrating the existence of nanoscopic and modulated liquid phase domains in a mixture composed entirely of nonhybrid lipids and cholesterol. Our results are indistinguishable from those obtained previously for mixtures containing hybrid lipids, conclusively showing that hybrid lipids are not required for the formation of nanoscopic liquid domains. These findings present new challenges for current theoretical descriptions of nanodomains.

2538-Pos Board B230

Characterization of Phosphatidylcholine/Tween-80 Model Membranes for the NMR Study of Membrane Interactions

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Tween-80 (TW80) is a fatty acid ester (oleate) of sorbitan polyethoxylate known to be a mild non-ionic surfactant and is commonly used to solubilize membrane proteins. In order to facilitate the structural study of membrane proteins and peptides by nuclear magnetic resonance (NMR), we have developed bicelle-inspired mimetics made of TW80. More specifically, the detergent was mixed with phosphatidylcholines (PCs) having different acyl chain lengths. The resulting PC/TW80 systems were characterized by solution- and solid-state (SS) NMR. Our results show that dimyristoylPC (DMPC, 14:0) and dipalmitoylPC (DPPC, 16:0) self-assemble with TW80 to form magnetically-oriented structures at PC/detergent molar ratios (q) of $5 \geq q \geq 2$ and $13 \geq q \geq 2$, respectively, as demonstrated by ^{31}P and ^{2}H SS-NMR. Oriented systems are formed above the gel-to-fluid phase transition temperatures of the phospholipids, i.e. 23°C for DMPC and 42°C for DPPC, up to high temperatures (77°C). ^{31}P solution NMR analysis demonstrates the formation of fast-tumbling isotropic structures at lower q ratios ($q<2$) for both PC/detergent membranes. The morphology and size of the PC/TW80 systems at various q ratios will be presented using dynamic light scattering data as well as cryo-electron microscopy and atomic force microscopy images. These new membrane mimetics should help studying membrane proteins and peptides by avoiding the detergent removal step during purification, and provide a new tool for the NMR study of membrane interactions.

2539-Pos Board B231

Kinetic Study of Photo-Induced Lipid Oxidation in Giant Unilamellar Vesicles

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Despite association of lipid oxidation to many key clinical concerns, the specific molecular mechanisms and dynamics of its roles in etiology and pathogenesis are not well understood. We have studied the kinetics of photo-induced phospholipid bilayer oxidation using giant unilamellar vesicles (GUVs) as model membranes. Incorporating rhodamine-labeled 1,2-dipalmitoyl-sn-glycero-3-phosphoethanoamine (Rh-DPPE) as photosensitizer to generate highly reactive singlet oxygen at the surface of 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC) GUVs, we observed several morphological changes during oxidation of the monounsaturated lipid bilayer. Within minutes of irradiation, the initial and brief growth of membrane area was accompanied by vesicle flattening and high-amplitude membrane fluctuations. After reaching a maximum area, the membrane began contracting, causing the GUVs to become tumid with a surface tension sufficient to exceed the critical limit for pore formation. The periodic micron-scale openings of pores could be quantitatively observed as step-decreases in vesicle size beyond pre-oxidation dimensions. The growth and shrinkage behaviors were analyzed as consecutive first-order rate-limiting steps within the overall oxidative process to estimate molecular size changes and the rate constants of the underlying chemical events. Dependence of these constants on irradiation intensity and on concentrations of Rh-DPPE and DOPC were considered. Furthermore, experiments varying membrane DOPC content were repeated at ten-fold increased aqueous viscosity to slow down fluid leakage and allow for more accurate measurement of pore closure rates. The calculated decreased line tension with higher DOPC concentration suggested pore edge-stabilizing geometry of oxidation products. In agreement with existing simulation results, we observed that photo-induced lipid oxidation consecutively expanded and reduced membrane area of DOPC GUVs, and our analysis suggested that these actions resulted from two key reaction steps occurring at different rates.

2540-Pos Board B232

Solubilization of Giant Vesicles Composed of Erythrocyte Lipid Extracts and of Ternary Lipid Mixtures by Triton X-100

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Detergents are widely used as solubilizing agents of biological membranes. Detergent resistant membranes, called DRMs, are frequently obtained and